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ORIGINAL ARTICLE

Co-administration of artemisinin and *Ricinodendron heudelotii* leaf extract—effects on selected antioxidants and liver parameters in male Wistar rats

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Abstract

Startling rate of malaria parasite resistance to artemisinin and its derivatives has led to possible herb–drug antimalarial combination therapy. This study assessed the effect of co-administration of artemisinin and *Ricinodendron heudelotii* extract on certain liver and antioxidant indices in rats. Four groups containing ten rats each were administered distilled water (group A), artemisinin only (group B), artemisinin with *R. heudelotii* extract (group C), and *R. heudelotii* extract only (group D). Serum biochemical values and antioxidant parameters were determined using standard methods respectively. The results revealed that the total protein level increased significantly ($p < 0.05$) in group C. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities increased significantly ($p < 0.05$) in the group administered artemisinin only but was regulated to control level both in groups C and D. The liver reduced gutathione (GSH) concentration decreased in the group administered artemisinin only. Similarly, malondialdehyde (MDA) level significantly increased ($p < 0.05$) in group A while groups C and D showed decrease in MDA and catalase concentrations. Histological examination showed that few of the hepatocytes were necrotic in the group administered artemisinin only while the group administered artemisinin and extract showed mild to moderate central venous congestion and periportal cellular infiltration. The study indicates that the bioactive constituents of the *R. heudelotii* extract might either have a regulatory effect on artemisinin toxicity or synergistically enhance its activity. Such bioconstituents can further be isolated and characterized for drug development to tackle *Plasmodium falciparum* resistance.

Keywords *Ricinodendron heudelotii* · Euphorbiaceae · Artemisinin · Antioxidants · Histology

Introduction

Malaria is one of the dominant communicable diseases with high incidence and mortality rate in developing countries. It is transmitted by anopheles mosquitoes that are infected with *Plasmodium* species. The greatest challenge of malaria therapy is that of resistance to antimalarial drugs such as chloroquine, sulfadoxine, and artemisinin (Ridley 2002). In addition, most of the antimalarial drugs have been reported to have high toxicity thus exposing patients' health to greater danger (Sullivan et al. 2011). This has made medicinal plants as potential targets for developing new drugs either as raw form or after synthetic formulation (Schmidt et al. 2012). Due to lack of effectiveness of most antimalarial drugs, traditional medicine used either solely or combined with orthodox medicines has become regular practice amongst people. This is prevailing in underdeveloped countries where the orthodox drugs are expensive to major part of the local community (Kremsner and Krishna 2004). Often times in West Africa, antimalarial traditional therapies are used simultaneously, before or after using these orthodox drugs. Minimal studies have been reported on how herbal medicines interact with the orthodox drugs. The possibility of herb–drug interactions (HDI) is

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hypothetically higher when compared to drug–drug interactions because it is believed that synthetic drugs comprise of individual chemical entities as opposed to herbs (Wanwimolruk and Prachayasittikul 2014). HDI can either alter the pharmacokinetic and/or pharmacodynamic properties of administered drugs; such pharmacokinetic interactions usually involve alterations in the gastrointestinal functions in relation to absorption, induction and/or the inhibition of some drug transporters and metabolizing enzymes, and change in drug excretion (Tarirai et al. 2010). These alterations can further result in synergistic, additive, and/or antagonistic effects (Fasinu et al. 2012). This raises a great concern as to how safe the concomitant use of herbal products with therapeutic drugs is to the body (Gorman 2012). Therefore, it is necessary to assess the interactions between herbs used in managing malaria and some standard antimalarial drugs. *Ricinodendron heudelotii* is a forest tree that is native to Central and West Africa and belongs to the Euphorbiaceae family (Clark and Sunderland 2004). It is commonly known as "Njansang" in Cameroun and "Okwe" in South East Nigeria (Izundu and Nnacho 2011). *R. heudelotii* has been used traditionally as a remedy against malaria amongst other uses (Fondoun et al. 1999). A decoction of the bark has been reported to exhibit aphrodisiac and diuretic effects in some areas of Cameroun (Momeni et al. 2005). Izundu and Nnacho (2011) reported that the seeds contain tannin, phenol, flavonoid, hydrogen cyanide, and saponin while unpublished data shows that the leaves contain flavonoids, terpenes, saponin, tannin, steroids, alkaloids, and cardiac glycosides. Hence, this study was designed to evaluate the effect of co-administration of the ethanol extract of *R. heudelotii* and artemisinin on some liver function and antioxidant indices in a rat model.

Methods

Drugs and chemicals

Artemisinin powder was purchased from Biotain Pharma Co. Ltd., China. Every other reagents were obtained from Sigma, USA, and they were of analytical grade.

Collection of plant

Ricinodendron heudelotii leaves were collected within the premises of Covenant University, Nigeria, and identified and authenticated by Dr. J.O. Popoola from the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria. Sample was deposited in the herbarium of the Forestry Research Institute of Nigeria, and voucher number FHI 110573 was obtained.

Extraction of plant

Powdered *R. heudelotii* leaves (1000 g) was extracted with 95% ethanol via maceration for 72 h and thereafter filtered and concentrated at 50 °C using a rotary evaporator (Adebayo et al. 2006) to obtain the ethanol extract having a yield of 16.25% (165 g).

Phytochemical study

The crude extract (150 g) was further extracted using ethyl acetate, hexane, and n-butanol in a separating chamber to obtain different fractions of the extract which was used for the quantitative and qualitative phytochemical screening following standard procedures. Phenolic content of the fractions was measured based on the Folin–Ciocalteu reagent reduction (Siddhuraju and Becker 2003) while measurement of the flavonoid content which was based on the principle that flavonoids form complexes with aluminum chloride was expressed as rutin equivalent obtained from a standard curve at different concentrations of rutin (Zhishen et al. 1999). The percentage alkaloid was also estimated according to the method of Harborne (1973).

Experimental animals

Forty male (120–150 g) Wistar rats used were obtained from the animal breeding center of the Federal University of Technology, Abeokuta, Ogun State, Nigeria. They were housed in standard cages under ideal conditions of 12/12 h dark/light cycle, 65% humidity, and 25 °C in the animal house of Biological Science Department. Animals were given daily diet and water ad libitum. This study was approved by the Biological Sciences Research Ethics Committee, Covenant University. All animals were also treated following the National Institutes of Health (NIH) guidelines for the usage and care of laboratory animals (NIH 2011).

Drug administration and experimental design

Animals were grouped into four as follows: Group A (control) received 0.1 ml of distilled water; group B were orally administered 2 mg/kg body weight of artemisinin only; group C received *R. heudelotii* extract (500 mg/kg) and artemisinin (2 mg/kg) separately and simultaneously; and group D received *R. heudelotii* extract only (500 mg/kg). All treatments were orally administered two times daily for 3 days.

Blood collection and sample preparation

The rats were sacrificed after treatment using diethyl ether as anesthesia, and blood collected from the heart into sample bottles containing heparin anticoagulant and centrifuged for

10 min at 3000 rpm to obtain the plasma used for the biochemical assays. Liver tissues were collected and homogenized using potassium chloride phosphate buffer [10 mM] with ethylene-diamine tetra acetic acid (EDTA, pH 7.4) and thereafter centrifuged for 15 min at 12,000 rpm. Catalase (CAT), malondialdehyde (MDA), and reduced glutathione (GSH) concentrations were assayed using the liver homogenate while some portion of the liver was excised for histopathological analysis.

Biochemical analysis

Test kits for the liver function test were purchased from Randox Laboratory, UK, and used for the biochemical analysis. Established procedures were used to evaluate alkaline phosphatase (ALP) (Tietz et al. 1983), aspartate aminotransferase (AST) (Bergmeyer et al. 1986a), total protein concentration (Weichselbaum 1946), and alanine aminotransferase (ALT) (Bergmeyer et al. 1986b).

Determination of CAT, GSH, and MDA in liver homogenate

CAT activity was determined following the method of Claiborne (1986) by observing the disappearance of hydrogen peroxide (H_2O_2) at 240 nm while GSH was determined following Ellman's method (Ellman 1959). MDA concentration was measured according to Buege and Aust (1978).

Histopathological analysis

Method by Aliyu et al. (2007) was used to carry out the histological analysis on the liver tissues.

Statistical analysis

All data were represented as mean ± SEM and analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test via the statistical package for social sciences (SPSS), version 21.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was taken at $p < 0.05$.

Results

Phytochemical analysis

Qualitative phytochemical analysis (Table 1) showed that the crude extract contains tannins, saponin, flavonoids, alkaloids, terpenoids, steroids, and quinone. The quantitative phytochemical screening shows the hexane and ethyl acetate fractions contain 7.70 ± 0.82 and 38.28 ± 0.61 mg GAE/g of phenol. The percentage alkaloid in the crude ethanol, ethyl

Table 1 Qualitative phytochemical screening of fractions of *Ricinodendron heudelotii*

Fractions/phytochemicals	CHO	Cardiac glycosides	Tannin	Saponin	Flavonoids	Alkaloids	Anthocyanin	Betacyanin	Quinone	Glycosides	Terpenoids	Triterpenoids	Phenol	Coumarin	Steroids
Crude	−	+	+	+	+	+	−	−	+	−	+	−	−	+	+
Hexane	−	−	−	+	−	−	+	+	−	−	−	−	+	−	−
Ethyl acetate	−	−	−	−	−	+	+	+	−	−	−	−	+	+	+
Butanol	−	−	+	+	+	+	+	+	+	−	+	−	−	−	+
Water	−	−	+	+	+	−	−	−	+	−	+	−	−	−	−

+ present, − absent

acetate, and butanol are 16, 12, and 20% respectively while the total flavonoids measured in the crude ethanol, butanol, and water fractions are as follows: 0.16 ± 0.005, 0.20 ± 0.013, and 0.23 ± 0.044 mg RE/g respectively (Table 2).

Antioxidant studies

The concentrations of MDA were significantly ($p < 0.05$) increased in the artemisinin only group while the catalase activity significantly reduced across all treatment groups. The GSH activities slightly increased in the combined treatment group (Fig. 1).

Biochemical studies

The activities of the aminotransferase (ALT and AST) significantly ($p \leq 0.05$) increased in the artemisinin only group. There was however a significant decrease in the combined treatment and extract only group, although there was an increase in the ALP level in the combined group which was not significantly different from the control group. The levels of total protein significantly increased in the combined and extract only group (Fig. 2).

Histological studies

Histological assessment of the liver tissues showed a moderate to severe diffuse vacuolar degeneration of hepatocytes (black arrows). Few hepatocytes are necrotic (red arrows) in the artemisinin only group while the group administered artemisinin and extract only showed mild to moderate central venous congestion and periportal cellular infiltration (Fig. 3).

Discussion

The startling rate at which malaria parasite counteracts the effect of malaria drugs has led to the search for more antimalarial compounds in medicinal plants especially in areas where medicinal plants are mostly used to treat or cure the disease; hence, additional investigation on plants must be embarked upon in order to conquer resistance in malaria therapy.

Artemisinin and its semi-synthetic derivatives have been reported to possess the swiftest action against *Plasmodium falciparum* (White 1997). Artemisinin which is obtained from *Artemisia annua* plant has also been reported to have reproductive and neurotoxic effect (Clark et al. 2008). Although they have been used in treating malaria, high cost of the drug, low bioavailability, and poor pharmacokinetic properties have been a draw back to its use; this has led many to combine the orthodox drug with herbal plant with the motive of it producing higher therapeutic effect ignoring the fact that it can lead to some unfavorable herb–drug interactions. Hence, this study assessed the possible effect of the concomitantly administering *Ricinodendron heudelotii* extract and artemisinin on certain liver and antioxidant indices in albino Wistar rats. The result obtained revealed an increase in total protein concentrations although this was only significant ($p < 0.05$) in the combined and extract only group (Fig. 2b). Such an increase might be as a result of the potential of artemisinin to boost protein synthesis (Udayashekara 1987). This correlates with previous work carried out by Adedosu et al. (2015).

The effect of artemisinin on certain liver enzymes indicated that artemisinin significantly increased ($p < 0.05$) activities of the aminotransferase (ALT and AST) in comparison with the control (Fig. 2a, b). The rise in the enzymes' activities in the group administered artemisinin only depicts liver injury due to increased membrane permeability and cellular necrosis as shown in the histopathological result (Fig. 3). Hence, the more porous the cell membrane, the more there will be a leakage of the intracellular enzymes into the circulating blood. This is in line with previous reports (Farombi et al. 2000; Li et al. 2006; Obi et al. 2004). Reports showed that drugs such as amodiaquine, chloroquine, and quinine elevate ALT and ALP levels and may induce liver damage (Udobre et al. 2009). Medicinal plants possess hepatoprotective effects due to the presence of some bioactive constituents (Subramanion et al. 2012). *R. heudelotii* leaf extract contains tannins, saponins, flavonoids, alkaloids, terpenoids, steroids, and quinine (Table 1). Flavonoids and tannins have been shown to have antioxidative activity and hepatoprotective and free radical scavenging capacity (Shashank and Abhay 2013; Gülçin et al. 2010). Saponin contained in some herbal plants has been reported to prevent hepatic and terminate cell proliferation (Lipkin 1995; Effiong and Akpan 2015); this effect was seen

Table 2 Quantitative phytochemical screening

Fractions	Total phenol (mg GAE/g)	% alkaloid	Total flavonoid (mg RE/g)
Crude	–	16	0.16 ± 0.005
Hexane	7.70 ± 0.82	–	–
Ethyl acetate	38.28 ± 0.61	12	–
Butanol	–	20	0. 20 ± 0.013
Water	–	–	0.23 ± 0.044

GAE gallic acid equivalent, *RE* rutin equivalent

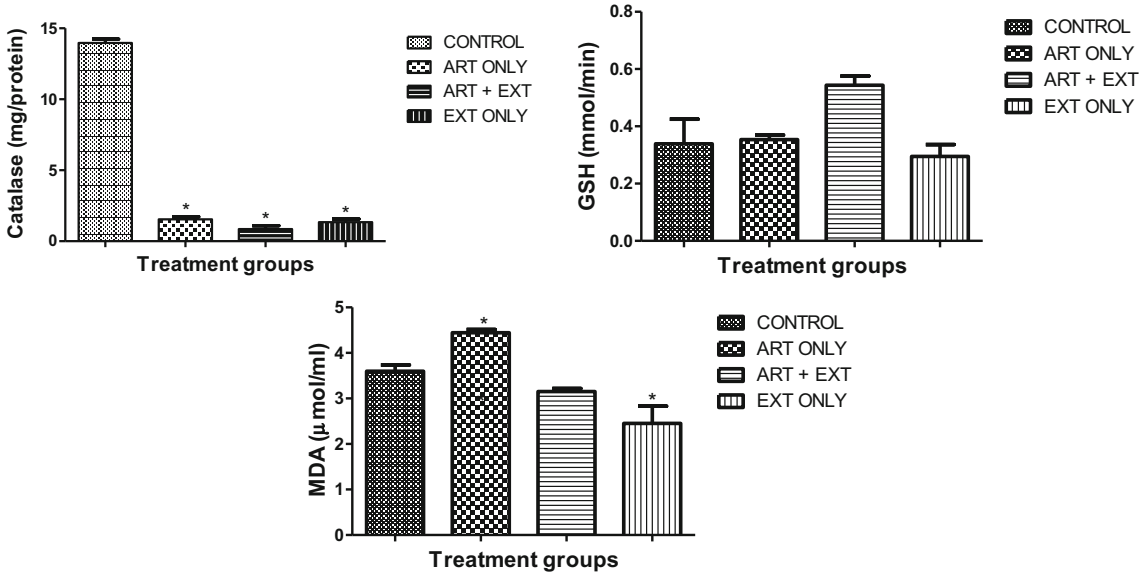

Fig. 1 Catalase, malondialdehyde (MDA), and reduced glutathione (GSH) concentrations in various treatment groups

in *R. heudelotii* leaf extract as it regulates the activities of the aminotransferases back to the control levels both in the combined and extract only treatment groups. This could also be accredited to the extract's ability to avert the artemisinin metabolism into harmful substances and thus reduce the release of free radicals further minimizing liver injury produced.

As lipid peroxidation is crucial in determining oxidative stress, significant rise in liver MDA levels in the artemisinin administered group (Fig. 1) suggests an increased lipid peroxidation leading to intense free radical production (Souza et al. 1997). Scavenging of free radicals is a major mechanism of antioxidation whereby lipid peroxidation reactions are inhibited. In this study, the ethanol extract of *R. heudelotii* both in the combined treatment group and when administered only decreased the MDA levels, indicating the antioxidant potential the plant extract to sustain membrane stability.

The primary function of reduced glutathione (GSH) is to detoxify various xenobiotics as well as scavenge free radicals (Prakash et al. 2004; Vudaa et al. 2012). The liver GSH concentration decreased in the artemisinin only group as compared to the other treatment groups (Fig. 1c). The combined treatment group however showed an increase in the liver GSH

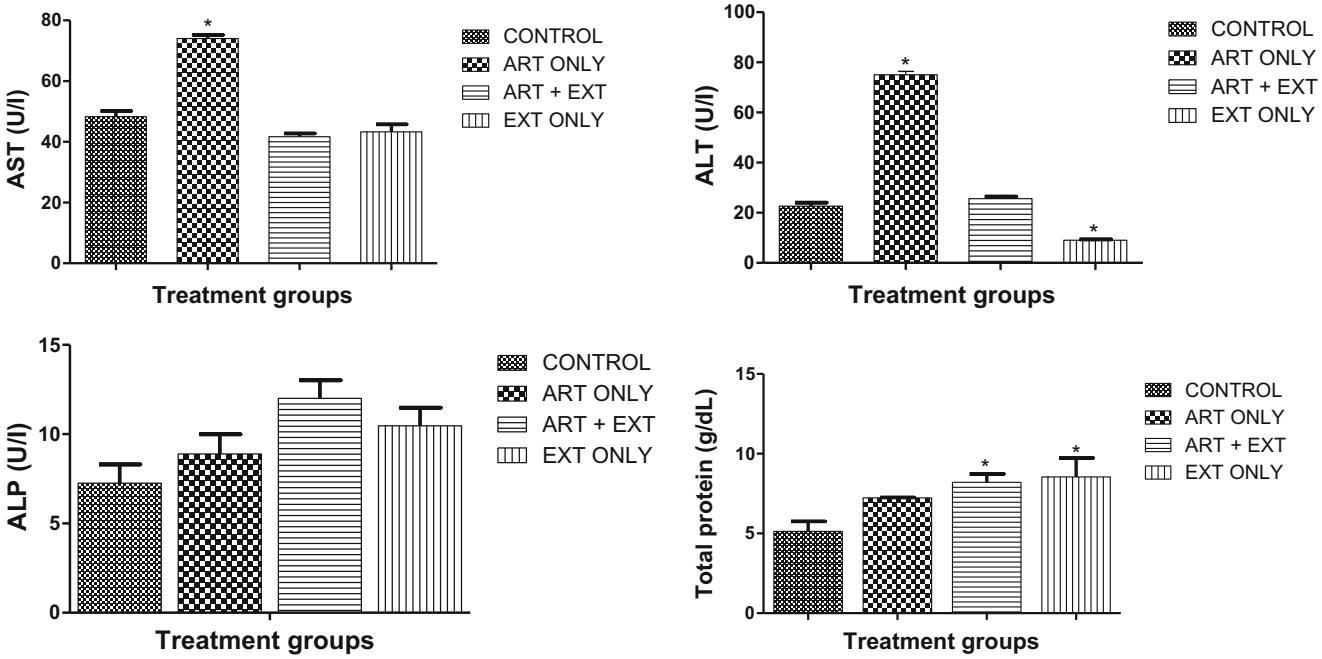

Fig. 2 Plasma aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), and total protein activities in various treatment groups

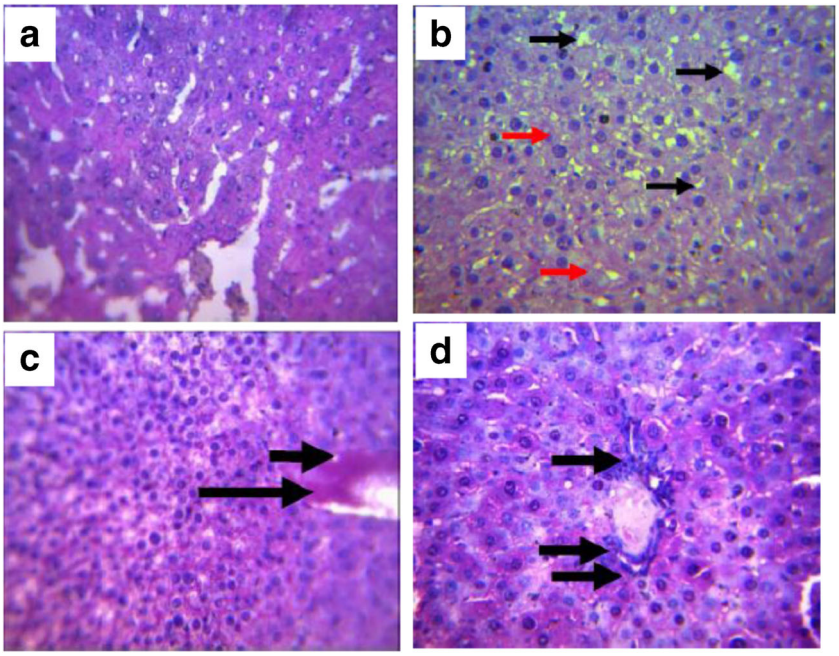

Fig. 3 **a** Photomicrograph of liver organ of rat with normal features in the control group, H&E × 400, **b** administered artemisinin only, **c** administered artemisinin and extract, and **d** administered extract only

concentration although not significant; this could also suggest the extract having antioxidant activity for hepatocellular defense against free radicals. Catalase is an antioxidant enzyme that spans across all animal tissues with highest activity in liver cells; it protects the cells from reactive hydroxyl radicals by putrefying hydrogen peroxide (Chance and Greenstein 1992). Artemisinin and extract both administered singly or in combined treatment decreased the catalase concentration either through oxidative inactivation or feedback obstruction of protein enzyme as a result of generation of excess reactive oxygen species (Guha et al. 2006). The reduction observed in catalase activity may lead to some adverse effects due to assimilation of hydrogen peroxide and superoxide radical. Qualitative phytochemical studies of *R. heudelotii* indicated the presence of saponins, flavonoids, alkaloids, cardiac glycosides, quinines, terpenoids, phenols, and steroids (Table 1) while from the quantitative screening, the extract showed a considerable amount of total phenol in the ethyl acetate and hexane fraction with the values of 38.28 and 7.70 mg/g gallic acid equivalence respectively while the flavonoid contents in the butanol and water fractions were 0.20 and 0.23 mg/g rutin equivalence. The presence of these compounds has been found to be predominant in plants with antioxidant properties thus having high impact on health (Dimitrios 2006). The quantitative phytochemical screening result correlates with the properties exhibited by the extract when administered in combination with artemisinin suggesting that the extract may have a different pharmacokinetic profile as an antioxidant in eliminating malaria parasite compared to artemisinin which induces oxidative stress to eliminate the parasite. This property may be explored as a clue to a possible mechanism to overcome resistance when the bioactive agents of this extract are used in combination with artemisinin.

Conclusion

Conclusively, results from this study show artemisinin administration led to rise in liver function and oxidative stress indices which was considerably lowered during the co-administration with *R. heudelotii* leaf extract, without causing any deleterious effect on animal organs. Hence, *R. heudelotii* leaf extract may have a regulatory effect on artemisinin toxicity. Thus, the bioactive constituents of the leaf can be explored for further research in overcoming drug resistance to *P. falciparum* malaria. Also, further research should be embarked upon to identify or isolate the active compounds responsible for these effects which could be helpful in drug design and development.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Informed consent Informed consent was obtained from all authors included in the study.

Ethical approval The research was approved by the Ethics Committee of the Department of Biological Sciences Covenant University, Nigeria. All animals were also treated in line with the National institute of Health

(NIH) guidelines for the use and care for animals in the laboratory (NIH 2011).

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